CLINICAL APPLICATIONS OF PHARMACOGENETICS

Clinical Pharmacokinetics (31:725:555)

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Goals/Objectives:

1. Review definitions of pharmacogenetics and pharmacogenomics and differentiate between pharmacogenetics and pharmacogenomics.
2. Outline relevant genetic terms.
3. Describe relevant SNPs and effects on therapy in phase I and II metabolism, transporters, and drug targets.
4. Highlight current and future uses for microarray monitoring.
5. Determine differences between genotype and phenotype.
6. Identify the CYP2D6 phenotype (Poor, Extensive, or Ultrarapid) of a patient based on given variants.
7. With a given phenotype of CYP2D6, predict the efficacy and ADR profile of propafenone, neuroleptics, tricyclic antidepressants, or metoprolol at normal doses.
8. Recognize pharmacogenetic alterations in drug-metabolizing enzymes and their implications.
9. Recall listed alterations in Phase II enzymes and their consequences.
10. Recognize drug target genes / receptors and their associated pharmacogenetic variance and clinical impact.
11. Apply understanding of discussed polymorphisms of drug targets to determine differences in other drug therapies.
12. Understand how pharmacogenetic information is obtained and how it may be used in the future.

"The future ain't what it used to be"—*Yogi Berra*

I. Definitions

A. Pharmacogenetics (PGt)

Search for genetic variation(s) that lead to inter-individual drug response (typically monogenetic) (i.e., the study of the relationship between individual gene variants and variable drug effects)

B. Pharmacogenomics (PGx)

The study of how genetic variations affect drug response. Evaluates the entire spectrum of genes that determine drug efficacy and safety (i.e., the study of the relationship between variants in a large collection of genes, up to the whole genome, and variable drug response)

1. Drug metabolizing enzymes
2. Drug receptors
3. Drug transporters
4. Drug targets

C. Purine Nucleotide Bases

1. Adenine (A)
2. Guanine (G)

D. Pyrimidine Nucleotide Bases

1. Cytosine (C)
2. Thymine (T)
3. Pair with purines to form base pairs
4. About 3 million base pairs in human genome

E. Codons

1. Three consecutive nucleotide base pairs that specify amino acids
2. 20 amino acids

F. Gene

1. Series of codons
2. About 30,000 genes in human genome

G. Alleles

- **Differences in the DNA sequence of the same gene**
- **The inherited maternal gene may have different SNPs than the inherited paternal gene; allele describes these differences**
- **Diplotype: refers to an allele pair, the allele inherited from each parent**

1. 2 at each gene locus (one from each parent)
2. 2 identical (homozygous)
3. 2 different (heterozygous)
4. May be reported as star-alleles (e.g., *CYP2D6*2)*
5. Star (*) alleles organize the different variants seen in the P450 system
6. Grouped according to functional effect and how commonly they are inherited together
7. Designations are based upon expert consensus

- The Human Cytochrome P450 (*CYP*) Allele Nomenclature Database (http://www.cypalleles.ki.se/)

II. Genetic Variations

A. Single nucleotide polymorphisms (SNPs)—a change in a single DNA nucleotide; most common variation

1. About 1 in 1000 base pairs

2. Wild-type

3. Variant
    a) homozygotes

    b) heterozygotes

4. Silent

5. May change protein expression

6. "Junk" DNA

B. Copy number variant

1.

B. Polymorphisms

1. At least 1% of population

C. Rare mutations (defects)

1. Less than 1% of population

D. A Genomic Biomarker

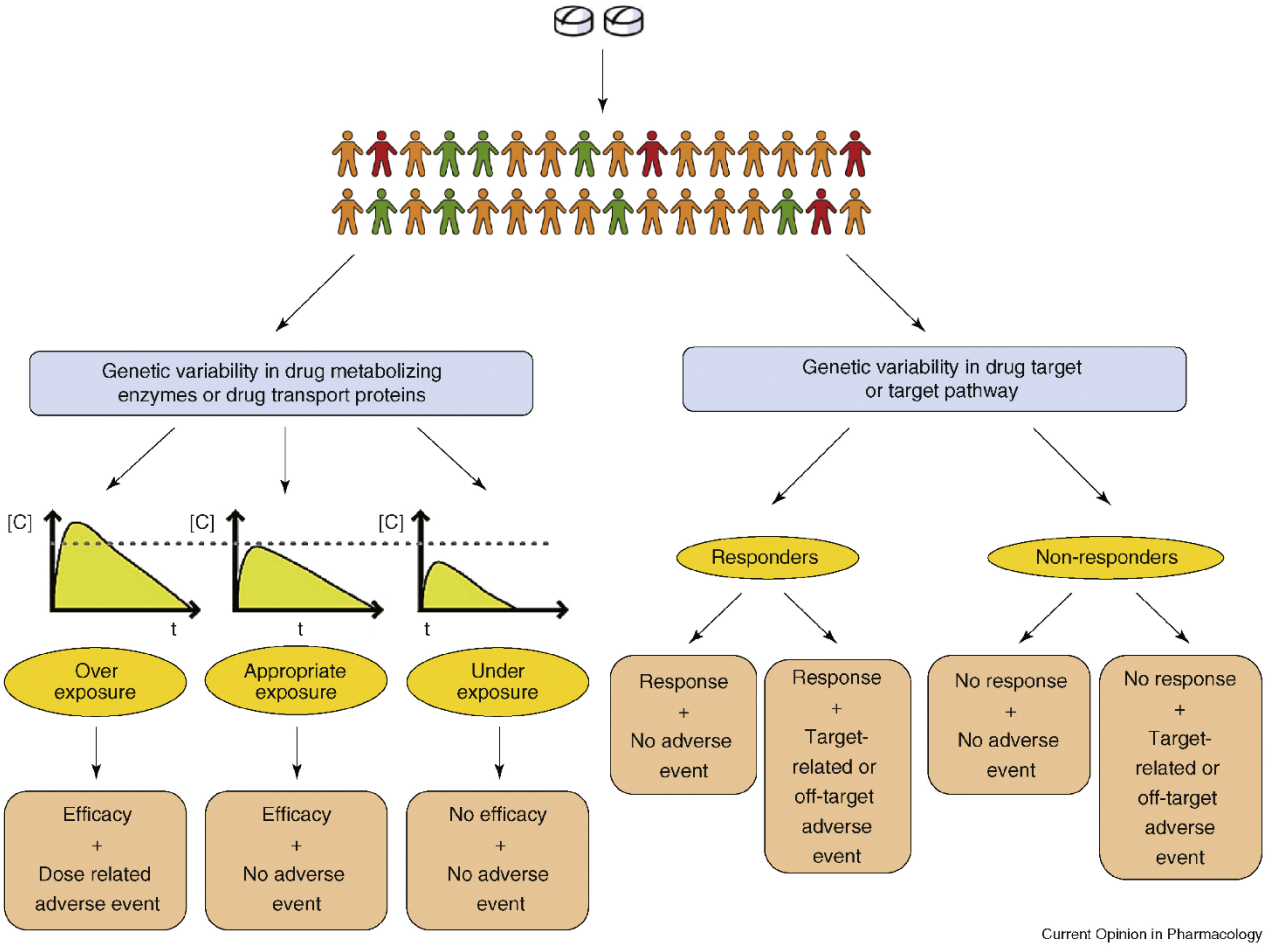
A measurable DNA and / or RNA characteristic that is an indicator of normal biologic processes, pathogenic processes, and / or response to therapeutic or other interventions

1. Expression of a gene
2. Function of a gene
3. Regulation of a gene

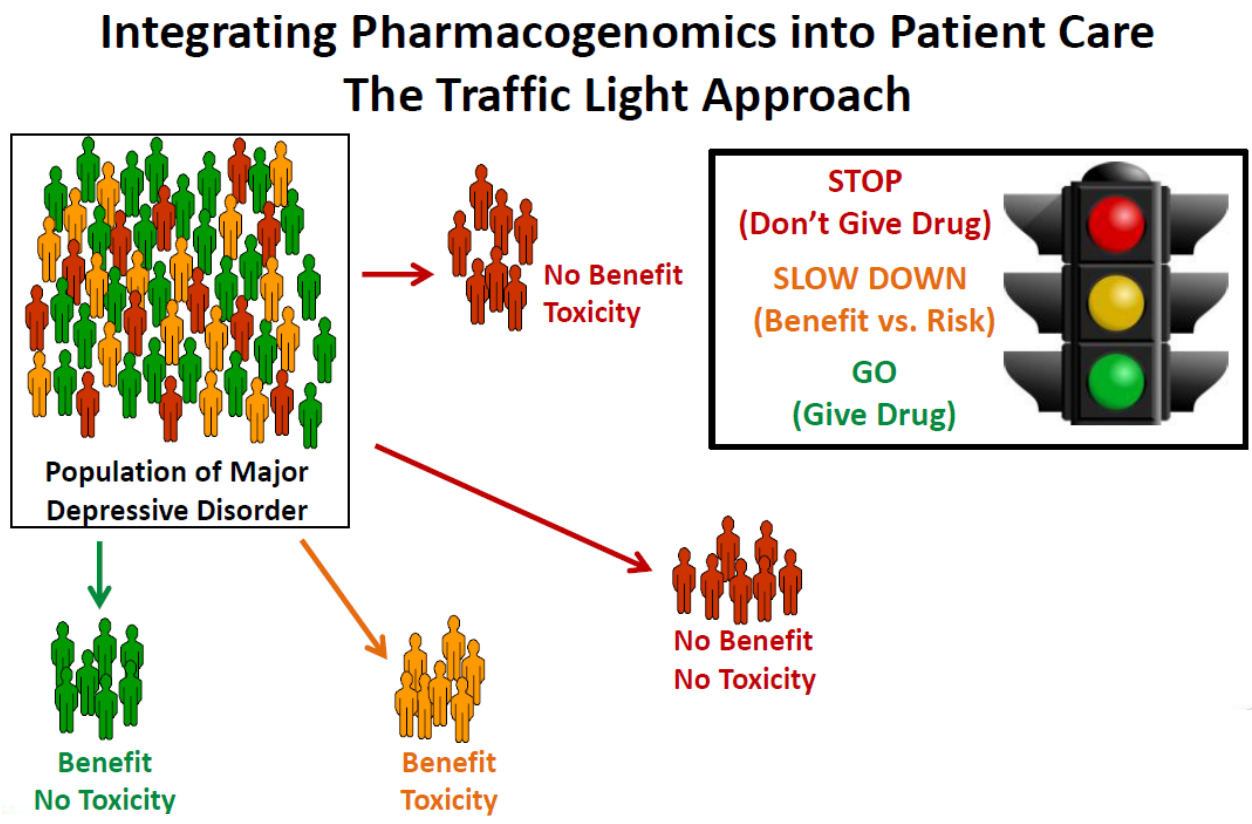
III. Standardizing Terms for Clinical Pharmacogenomic Test Results (consensus terms from the *Clinical Pharmacogenetics Implementation Consortium (CPIC)*)

Proposed terms for PGx Results (phenotypes)	
Drug Transporters	**Drug Metabolizing Enzymes**
• Increased function • Normal function • Decreased function • Poor function	• Ultrarapid metabolizer • Rapid metabolizer • Normal metabolizer • Intermediate metabolizer • Poor metabolizer
High-risk genotype	
• Positive • Negative	

Schematic Overview of Genetic Variability Affecting Drug Response and Disposition. ([C] = Drug Concentration and *t* = Time)



From: *Current Opinion in Pharmacology. 2008;8:639-646.*



From: *American Society of Health-System Pharmacists; Pharmacogenomics Education Series; Introduction to Pharmacogenomics (2016)*

IV. Drug-Metabolizing Enzymes

A. Phase I (oxidation)

1. CYP2D6

a) Best characterized

b) 48 (at least) variants defined

c) Poor metabolizer phenotype (PM)

- 2 defective alleles
  - 5 – 10% of Caucasians
  - 1 – 3% of African-Americans & Asians

d) Extensive metabolizer phenotype (EM) = "normal" = 'most common'

- 90% of Caucasians
- >98% of Asians & Native Americans

e) Ultra-rapid metabolizer phenotype (UM)

f) Phenotype predicted with 99% confidence by 6 variants

- CYP2D6*1 (wild-type) Normal Activity
- CYP2D6*2 (capable of duplication or amplification) Normal or Enhanced activity
- CYP2D6*4 (defective splicing) Inactive enzyme
- CYP2D6*5 (gene deletion) Absence of enzyme

- CYP2D6*10 (amino acid substituted) reduced activity. Observed in Asians
- CYP2D6*17 (amino acid substituted) reduced activity. Observed in African Americans

g) Ultrarapid metabolizers (UM) carry CYP2D6*2
h) PMs: Codeine and tramadol (prodrugs): reduced activation to active moiety
i.) UMs: Codeine: rapid, complete conversion to morphine, may result in higher than expected concentrations and related ADEs
j) PMs: Increased ADEs with propafenone, neuroleptics, and tricyclic antidepressants
k) UMs: require very high doses to achieve therapeutic levels
l) EMs taking a CYP2D6 inhibitor had increased metoprolol effect (not seen in PMs)
m) PMs: Tamoxifen: higher risk of breast cancer recurrence(?)
n) Atomoxetine
- PM: higher plasma concentrations vs. EMs

o) Fluoxetine: Inhibits CYP2D6 activity; thus, EMs may function as PMs

2. CYP2A6

a) Absence associated with inability to metabolize nicotine
b) Deletion common in Asian patients (20%) vs. European/Caucasians (<1%)

3. CYP2C9

a) Warfarin, phenytoin, tolbutamide, celecoxib
b) Decreased warfarin metabolism (clearance) and bleeding documented

Relationship Between S-Warfarin Clearance & CYP2C9 Genotype in Caucasian Patients

CYP2C9 Genotype	Mean S-Warfarin Clearance/Lean Body Weight (mL/min/kg)
*1/*1	0.065
*1/*2 or *1/*3	0.041
*2/*2 or *2/*3 or *3/*3	0.02

Adapted from the Coumadin® (warfarin) package insert, rev. January 2010

c) Celecoxib
- PM: Increased plasma concentrations due to reduced clearance

4. CYP2C19

a) Clopidogrel, lansoprazole, omeprazole, phenytoin, voriconazole
b) PM common in Asians & African-Americans (15-20%)
c) PM: 3 – 5% of Caucasians
d) PM increased omeprazole AUC 12-fold vs EM

e) *H. pylori* cure with omeprazole + amoxicillin
- PM: 100% cure rate
- Heterozygous EM: 60% cure rate
- Homozygous EM: 29% cure rate

f) Voriconazole
- EM (homozygous): "normal" voriconazole plasma concentration
- EM (heterozygous): 2-fold higher voriconazole exposure than homozygous EM
- PM: 4-fold higher voriconazole exposure than homozygous EM

g) Clopidogrel
- Involved in the formation of the active & intermediate metabolites
- PM: associated with diminished response to clopidogrel

5. CYP3A4 / CYP3A5

a) Variants have been identified
b) Phenotype poorly characterized currently
c) Clinical applicability is evolving
d) CYP3A5 polymorphism associated with increased tacrolimus dosing requirements

B. Phase II (conjugation)

1. Atypical pseudocholinesterase

a) Beginning of pharmacogenetics
b) Exaggerated and prolonged response to succinylcholine

2. Glutathione S-transferase

a) PM associated with hemolytic reactions with primaquine

3. Thiopurine S-methyltransferase (TPMT)

a) PM: 1 in 300 patients
b) Severe leukopenia/death on azathioprine or mercaptopurine

4. N-Acetyltransferase (NAT)

a) Slow acetylators: Higher concentrations; increased ADRs
- Hydralazine: drug-induced lupus
- Isoniazid: peripheral neuropathy

5. UDP-glucuronosyl transferase (UGT) 1A1

a) Irinotecan
- PM (homozygous): increased risk of neutropenia and diarrhea due to decreased inactivation; reduced initial dose may be indicated
- PM (heterozygous): increased risk of neutropenia, but clinical results are variable

b) Nilotinib
- (TA)7/(TA)7 genotype associated with a statistically significant increase in the risk of hyperbilirubinemia relative to other genotypes

6. Dihydropyrimidine Dehydrogenase (DPD)

a) Capecitabine, 5-fluorouracil
- DPD deficiency is associated with severe toxicity (stomatitis, diarrhea, neutropenia, neurotoxicity)

7. Glucose-6-phosphate dehydrogenase (G6PD)

a) Dapsone
- G6PD deficiency is associated with an increased risk of severe hemolytic anemia

V. Transporters

A. P-glycoprotein (MDR-1 gene product)

1. Appears in many tissues to protect the body from toxins
2. Increased expression decreases drug concentrations (e.g., digoxin)
3. Decreased expression increases drug concentrations
4. At least 15 polymorphisms
5. Africans appear to have highest expression

VI. Drug Target Genes (Receptors)

A. Renin-Angiotensin-Aldosterone System

1. ACE
2. Angiotensinogen
3. Bradykinin
4. Conflicting data across various uses of ACEI, may relate to influence of multiple rather than single SNPs

B. Beta-receptors

1. May explain ethnic differences in BP response to beta-blockers
2. $Beta_2$-gene
    a) > 10 SNPs identified
    b) May affect bronchodilation response from albuterol

C. Lipoxygenase

1. Variants have reduced increase in FEV_1 to 5-lipoxygenase inhibitor

D. Vitamin K epoxide reductase complex, subunit 1 (*VKORC1*)

1. Warfarin
2. Polymorphism associated with lower dosage requirement
3. Combination of factors explains variability in dose
    a) 30% of variability: VKORC1 gene alone
    b) 40% of variability: VKORC1 + CYP2C9 genes combined
    c) 55% of variability: VKORC1 + CYP2C9 + age, height, body weight, interacting drugs, and indication for warfarin

Three Ranges of Expected Maintenance Warfarin Daily Doses
Based on CYP2C9 and VKORC1 Genotypes†

VKORC1	CYP2C9					
	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3
GG	5-7 mg	5-7 mg	3-4 mg	3-4 mg	3-4 mg	0.5-2 mg
AG	5-7 mg	3-4 mg	3-4 mg	3-4 mg	0.5-2 mg	0.5-2 mg
AA	3-4 mg	3-4 mg	0.5-2 mg	0.5-2 mg	0.5-2 mg	0.5-2 mg

†Ranges are derived from multiple published clinical studies. VKORC1 –1639G>A (rs9923231) variant is used in this table. Other co-inherited VKORC1 variants may also be important determinants of warfarin dose.

Adapted from the Coumadin® (warfarin) package insert, rev. October 2011

E. Human Leukocyte Antigen-B*5701 (*HLA-B*5701*)

1. Abacavir (abacavir hypersensitivity reaction [ABC-HSR])
    a) Marker for ABC-HSR (potentially life-threatening)
    b) ABC-HSR characterized as ≥ 2 of the following symptom categories
        Group 1: Fever
        Group 2: Rash
        Group 3: N/V/D or abdominal pain
        Group 4: Generally ill feeling, extreme tiredness, or achiness
        Group 5: Shortness of breath, cough, or sore throat
    c) *HLA-B*5701* screening

F. Human Leukocyte Antigen-B*1502 (*HLA-B*1502*)

1. Carbamazepine (? Phenytoin and fosphenytoin ?)
    a) Marker for dangerous or even fatal skin reactions:
        - Stevens Johnson syndrome
        - Toxic epidermal necrolysis
    b) Prevalence of allele as high as 15% in those with Asian ancestry

G. Human Leukocyte Antigen-DQB1 (*HLADQB1*)

1. Clozapine (clozapine-induced agranulocytosis [CIA])
    a) 2 single-nucleotide polymorphisms to characterize risk of CIA relative to untested population
    b) Higher risk: 2.5 relative risk of CIA
    c) Lower risk: 0.5 relative risk of CIA

H. Organic Anion Transport Protein 1B1 (*SLCO1B1*)

1. Variants associated with increased susceptibility to statin-induced myopathy

I. Many others documented

1. Cholesterol pathway
2. Neurotransmitter receptors

VII. Obtaining and Using Genetic Information

A. Retrospective Analysis

1. Most data from observing ADRs or therapeutic effect differences
2. Genetics to explaining differences (Phase IV marketing)

B. Prospective

1. Including pharmacogenetic/genomic aspects in Phase I-III trials
2. Some cancer centers test TPMT prior to administering therapy

C. Microarray Analysis

1. Screening
    a) proto-oncogenes: BRCA1, BRCA2, p53, HER2
    b) Clinical screening for BRCA mutations

2. Treatment
    a) CYP, Phase II, and transporter SNPs
    b) HER2 for trastuzumab (not quite pharmacogenetics/genomics) since identifying different protein expression rather than a genetic polymorphism

D. *AmpliChip CYP450 Test*

1. Blood-derived DNA
2. Detect gene variations and determines metabolizer status
    a) CYP2D6
    b) CYP2C19

E. Invader *UGT1A1* Molecular Assay

1. Detects specific gene mutations in the *UGT1A1* gene associated with ADRs to irinotecan
2. May assist with individualizing drug doses

F. Factors influencing the clinical utilization of pharmacogenetic testing/screening

1. Availability of testing (commercial, clinical, research, home-grown, etc.)
2. Turn-around-time for results
3. Reproducible / valid results
4. Cost-effectiveness
5. Health care provider education
6. Bioethics [Genetic Information Nondiscrimination Act (GINA)]
7. *The Immortal Life of Henrietta Lacks*, Rebecca Skloot

VIII. ASHP Statement on the Pharmacist's Role in Clinical Pharmacogenomics (Am J Health-Syst Pharm 2015; 72: 579-81)

ASHP POSITION: Pharmacogenomics (PGx) can improve medication-related outcomes across the continuum of care

A. ASHP recommendations for all pharmacists' functions

1. Recommend PGx resting when appropriate
2. Design patient-specific medication regimens based on patient characteristics including PGx
3. Educate patients and clinicians about PGx
4. Communicate PGx-specific drug therapy recommendations to the health care team

IX. Pharmacogenomics resources relevant to pharmacists (Am J Health-Syst Pharm 2015; 72:1324-8)

Source	Resource Highlights	Website
Pharmacogenomics Knowledge Base	Comprehensive resource that curates knowledge about the impact of genetic variation on drug response for clinicians and researchers (more into research)	www.pharmgkb.org
Genetics and Genomics Competency Center	Created to provide healthcare professionals with a learning management system designed to enhance efforts to develop transdisciplinary approaches to genetics/genomics education (relevant for more basic genomics principles)	http://g-2-c-2.org/
Clinical Pharmacogenetics Implementation Consortium	Provides guidelines that enable the translation of genetic laboratory test results into actionable prescribing decisions for specific drugs (most focused on pharmacogenomics)	www.pharmgkb.org/page/cpic
PharmGenEd	An evidence-based program designed for pharmacists and physicians, pharmacy and medical students, and other healthcare professionals	https://pharmacogenomics.ucsd.edu/
St. Jude Children's Research Hospital	Institutional resource providing links to publications, video vignettes, and regulatory information	www.stjude.org/pg4kds/implement
University of Florida College of Pharmacy	Comprehensive resource providing links to topical research articles, a serial newsletter free for subscribers called *SNPits*, and information regarding clinical practices affiliated with Shands Medical Center	http://personalizedmedicine.ufhealth.org/snp-its/about-us-2/subscribe/
Food and Drug Administration	Center for Drug Evaluation and Research home page with links to clinical pharmacology resources, drug labels with genetic information and recommendations, and other drug information resources	www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/